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Bioavailability of Volatile Organics and Other Hydrocarbons from Environmental Media: Ingestion in Drinking Water

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19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The primary objectives of this project were to: (a) assess the roles of hepatic and pulmonary presystemic elimination in reducing the bioavailability of low levels of volatile organic chemicals (VOCs) found in drinking water supplies; (b) investigate gastrointestinal (GI) absorption pathways for VOCs; (c) characterize the influence of oil dosage vehicles on the absorption, pharmacokinetics (PK) and toxicity of VOCs, with emphasis on potential mechanisms by which corn oil acts. Substantial progress has been made towards achieving each of these objectives. Studies in unanesthetized, male Sprague-Dawley rats, contrasting the PK of equal doses of VOCs given orally as a single bolus and by constant intragastric (ig) infusion for up to 6 hours, revealed significantly lower peak blood levels and bioavailability in the ig groups. Blood concentrations of well metabolized VOCs, such as trichloroethylene (TCE) and 1,1-dichloroethylene, were so low that they were hardly detectable at low dosage levels in the ig animals. These findings suggest that the liver and lungs may be able to remove virtually all of the trace amounts of VOCs that are usually found in drinking water.

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As anticipated, the toxicity of a potent hepatotoxin, carbon tetrachloride (CCl_4), was considerably lower when the chemical was given to rats by gastric infusion than when given as an oral bolus. The influence of route of exposure on the pharmacokinetics and hepatotoxicity of CCl_4 was also assessed as a part of the foregoing study. Groups of rats were administered equal doses of CCl_4 by inhalation and by ig infusion over a 2-hour period. Serial blood samples were taken for up to 12 hours during and post exposure, and analyzed for their CCl_4 content by headspace gas chromatography. Peak arterial blood levels and bioavailability of CCl_4 were significantly greater in the inhalation groups, indicative of a high capacity for hepatic uptake/metabolism of the VOC. Experiments were performed to accurately determine the relative contribution of the liver and lungs, as well as the (maximum) capacity and dose-dependency of presystemic elimination of TCE in rats. Total presystemic elimination was inversely related to dose, ranging from >70% at 0.3 mg TCE/kg bw, to <10% at 13.6 mg/kg and higher. The fraction eliminated by the lungs (10-16%) remained constant over the range of doses, while the fraction eliminated by the liver increased as the dose decreased. First pass hepatic elimination accounted for ~51% of the lowest doses. Preliminary experiments indicate that an even higher percentage of TCE will be eliminated before it enters the arterial circulation, if is ingested over a prolonged period (as typically occurs under actual environmental exposure conditions). The role of the lymphatics in systemic absorption of ingested VOCs has also been studied. A surgical technique was developed which allowed collection of lymph from the thoracic duct of unanesthetized rats. Experiments indicated that CCl_4 was absorbed to a limited degree via the lymphatics when given orally in an aqueous emulsion, but that lymphatic absorption was much greater when the chemical was administered in corn oil. Other studies in rats revealed that corn oil markedly delayed and prolonged the GI absorption of CCl_4, thereby diminishing its acute hepatotoxicity. A system analysis approach was successfully utilized in conjunction with a physiologically-based pharmacokinetic model to simulate the oral absorption of CCl_4 given in a corn oil vehicle. Subacute studies revealed that CCl_4 was somewhat more hepatotoxic when given for up to 4 weeks in corn oil, although there was little difference from an aqueous vehicle group after 13 weeks of exposure. Additional studies of potential mechanisms by which corn oil alters the PK and toxicity of CCl_4 have shown that corn oil dosing caused increased accumulation of triglycerides in the liver, which in turn resulted in greater deposition of CCl_4 in the liver. The high lipid intake did not result in increased lipoperoxidation, nor in an increase in hepatic microsomal cytochrome P-450 levels nor altered mixed-function oxidase (drug metabolism) activity. Dietary fatty acids were found to be necessary for optimal activity and induction of P-450 in rat liver. Experiments revealed that dietary fat deprivation did protect against CCl_4 hepatotoxicity, apparently by reducing levels of P-450 isozymes necessary for metabolic activation of CCl_4 to cytotoxic metabolites.

BIOAVAILABILITY OF VOLATILE ORGANICS AND OTHER HYDROCARBONS FROM ENVIRONMENTAL MEDIA: INGESTION IN DRINKING WATER

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I. OVERALL OBJECTIVE AND SPECIFIC AIMS

The OVERALL OBJECTIVE of the project was to critically evaluate and characterize the bioavailability of low levels of volatile organic chemicals (VOCs) present as contaminants of drinking water supplies in the United States.

SPECIFIC AIMS were to:

(1) Characterize and ascertain the significance of hepatic (i.e., liver) and pulmonary (i.e., lung) presystemic elimination of ingested VOCs. Studies were conducted to determine how effective presystemic elimination is in removing the small (trace) amounts of VOCs typically consumed in water.

(2) Investigate gastrointestinal (GI) absorption pathways. Studies were conducted to determine whether carbon tetrachloride, a short-chain aliphatic halocarbon, is absorbed from the GI tract enter the portal venous circulation, or bypasses the liver via the lymphatic system.

(3) Characterize the influence of oil dosage vehicles/diluents on the absorption, pharmacokinetics (PK) and toxicity of VOCs, as well as investigate potential mechanisms by which corn oil may affect the PK and toxicity of orally administered VOCs.

II. PROFESSIONAL PARTICIPANTS ON PROJECT

James V. Bruckner, Ph.D., Principal Investigator

Randall O. Manning, Ph.D., Investigator

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III. STATUS REPORT ON ACCOMPLISHMENTS AND PROGRESS MADE TOWARDS ACHIEVING RESEARCH OBJECTIVES AND SPECIFIC AIMS

A. Determination of the Influence of Dosage Regimen and Exposure Route on the Pharmacokinetics and Toxicity of VOCs

The OBJECTIVE of this phase of the project was to examine the applicability of oral bolus studies to the assessment of health effects of drinking water contaminants. Although VOCs and other organic chemicals are usually given by gavage (orally) as a single bolus, human exposures typically occur on a repetitive or continuing basis, as a person consumes water over the course of the day. Single oral bolus doses of VOCs should produce relatively high blood and target organ levels of the chemicals. It appears likely that bolus doses can cause toxicity by exceeding the capacities of presystemic elimination and of defense systems in target tissues. In contrast, the relatively small quantities of these chemicals absorbed in divided doses should be extensively metabolized and/or exhaled, such that toxic levels may not be reached in tissues. If this HYPOTHESIS is true, toxic effects of VOCs in most target organs should be

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significantly less pronounced under actual environmental exposure conditions than in experiments in which the chemicals are given once daily as an oral bolus.

INHALATION of VOCs should result in higher levels of the chemicals in arterial blood and extrahepatic organs (and therefore greater toxicity) than equivalent ORAL exposures. Chemicals absorbed in the lungs directly enter the arterial circulation, whereas chemicals absorbed from the GI tract into the venous blood must first pass through the liver and lungs before reaching the systemic circulation. Thus, it can be HYPOTHESIZED that the liver may effectively remove VOCs from the venous blood, but therefore be at greater risk of toxic injury (upon oral exposure). The liver should be most effective at removing relatively small amounts of VOCs absorbed into the blood following low doses (i.e., doses that do not exceed the metabolic capacity of the liver).

We have conducted three series of experiments to assess the influence of PATTERN and ROUTE of exposure on the pharmacokinetics (PK) and target organ toxicity of carbon tetrachloride (CCl_4). In the first study, male Sprague-Dawley (S-D) rats inhaled 100 or 1,000 ppm CCl_4 for 2 hours through a one-way breathing valve. The administered dose was estimated using the following equation: 0.5 X respiratory minute volume X time X inhaled concentration. The factor of 0.5 was utilized as it was assumed, based on the known valvular, mask and anatomical dead-space, that 50% of the inhaled CCl_4 actually participated in systemic uptake. The equivalent doses to administered orally were thus calculated to be 18.9 and 186 mg/kg, for the 100 and 1,000 ppm exposures, respectively. The oral doses of CCl_4 were given to rats either as a single bolus or via a surgically-implanted gastric cannula as an aqueous Emulphor® emulsion over 2 hours by constant intragastric (ig) infusion. We felt that ig infusion was the most appropriate way to administer CCl_4 in the "route-to-route" studies, since the same dose of chemical could be given over the same time-frame (i.e., 2 hours). Serial blood samples were taken from the subjects and analyzed for CCl_4 by headspace gas chromatography (GC), in order to obtain blood concentration versus time profiles. Blood and liver specimens were taken 24 hours post exposure for measurement of serum enzyme levels and hepatic microsomal enzymes, which were used as indices of liver injury.

In the initial experiment maximum blood levels (C_{max}), area under the blood concentration vs. time curve (AUC) and hepatotoxicity indices were substantially higher for the oral bolus than the corresponding ig group. These results demonstrate that the oral dosage regimen, or pattern of exposure can have a significant impact on toxicity test results. Furthermore, the findings are evidence that toxicity testing protocols should approximate actual exposure conditions as closely as possible. Inhalation resulted in significantly greater C_{max} and AUC values than did ig infusion at both dosage-levels. Extrahepatic organs (i.e., all organs of the body other than the liver) should thus receive higher doses of CCl_4 and many other volatile chemicals) and thus experience more pronounced toxic effects upon inhalation than upon ingestion. Our data indicate that the liver and lungs are quite efficient in removing CCl_4 from the portal blood, following its absorption from the GI tract (i.e., effective in presystemic elimination). The liver would appear to be a greater risk of injury, upon oral exposure, due to greater uptake of CCl_4. Our hepatotoxicity data, however, were not conclusive. There was little difference between the oral (ig) and inhalation groups in serum enzyme levels. The only index of liver injury showing a more

pronounced effect in the ig animals was microsomal P-450, which was lower in the oral than in the inhalation group at the high dosage level. It should be noted that the administered inhaled dose was utilized (given orally) here. Experiments were deemed necessary to determine the actual systemically absorbed dose during CCl_4 inhalation. Results of the foregoing experiments were presented at a national conference on risk assessment, which was cosponsored by the U.S. EPA and the International Life Sciences Institute (ILSI) in 1990, and published the same year (Bruckner *et al.*, 1990a).

A second series of experiments was conducted to further clarify the influence of ROUTE and PATTERN of exposure to VOCs on toxicity In this series, systemically absorbed doses in inhalation exposures were accurately measured, and these doses given to other groups of animals. Male S-D rats of 300-400 g were acclimated to cylindrical plastic restraining tubes of the type used in nose only exposures. These animals were subsequently placed into the tubes and fitted with rubber masks equipped with miniaturized one-way breathing valves. Once stable breathing patterns were established, the rats inhaled 100 or 1,000 ppm CCl_4 for 2 hours. Repetitive samples of the inhaled and exhaled breath streams were collected and analyzed for CCl_4 by GC. The systemic uptake (i.e., absorbed dose) of CCl_4 was calculated on the basis of the difference between the inhaled and exhaled CCl_4 concentration, the respiratory rate and volume and the deadspace volume. The systemically absorbed doses for the 2-hour 100 and 1,000 ppm exposures were determined to be 17.5 and 179 mg/kg bw, respectively. It should be noted here that these values are not very different from our prior estimates (i.e., 18.9 and 186 mg/kg bw) of doses of CCl_4 administered under similar inhalation exposure conditions (Bruckner *et al.*, 1990a).

In the second series of experiments, other groups of rats were given the 17.5 and 179 mg/kg doses: (a) as a single (oral) bolus in a 1% Emulphor® aqueous emulsion; (b) by constant gastric infusion over a 2-hour period via a gastric cannula. Serial micro-blood samples were collected from an indwelling right carotid artery cannula at intervals of 2 to 60 minutes for up to 12 hours, in order to obtain complete blood CCl_4 concentration versus time profiles. Lost blood volume (total ~1.5 ml) was replaced with physiological saline. Twenty-four hours post exposure, each animal was etherized and blood collected by cardiac puncture for measurement of serum sorbitol dehydrogenase (SDH) and glutamic-pyruvic transaminase (GPT) activities. Liver samples were taken and the microsomal fractions isolated for measurement of cytochrome P-450 levels and glucose-6-phosphatase (G-6-Pase) activity. These two parameters, as well as SDH and GPT activities, are sensitive and specific indices of liver damage.

Findings in the second series of experiments were similar in many respects to those in the first study. CCl_4 was rapidly absorbed from both the lungs and GI tract. Peak blood levels (C_{max}) were reached within 10-15 minutes of oral bolus dosing. C_{max}s in the two oral bolus groups were 30 times higher than those in the respective gastric infusion groups. AUC values were substantially greater in the oral bolus groups, indicating greater bioavailability of CCl_4 to tissues throughout the body. Thus, one would anticipate that oral bolus dosing would produce more pronounced organ damage. Indeed, CCl_4 was significantly more hepatotoxic (as manifest by increases in SDH and GPT, and a decrease in cytochrome P-450 levels) when given as a single oral bolus. These findings indicate that oral bolus dosing regimens routinely used in toxicity studies are

NOT representative/valid means of assessing the toxic potential of chemicals encountered environmentally.

Results in the second series of experiments may be used to assess the influence of ROUTE of exposure on the pharmacokinetics and hepatotoxicity of CCl_4. Blood CCl_4 concentrations (including C_{max}s) in the inhalation groups were markedly higher than in the corresponding gastric infusion groups during the 2-hour exposures, indicating greater delivery of the chemical to extrahepatic organs. However, after cessation of exposures, the blood levels in the inhalation groups fell more rapidly than in the gastric infusion groups. The terminal elimination half-lives in the latter groups were significantly longer. Consequently, AUC values were not significantly different in the inhalation and gastric infusion groups. CCl_4 appeared to be slightly more hepatotoxic when administered by inhalation, although only G-6-Pase was significantly altered. Two observations may be important here: (a) CCl_4 concentrations during peak/relatively early times post exposure appear to be most important in hepatotoxicity (Kim *et al.*, 1990a); and (b) CCl_4 may redistribute over time following dosing, such that initial route-dependent differences may be obscured. The aforementioned experiments were published in abstract form (Sanzgiri *et al.*, 1991a) and included in a manuscript submitted for publication (Sanzgiri *et al.*, 1992a).

As blood levels of a chemical are only an indirect measure of target organ levels, a third series of experiments was undertaken to establish time-courses of tissue uptake of CCl_4 and hepatotoxicity in animals given CCl_4 orally. As related above, subsequent studies will need to be conducted to elucidate the relative uptake and toxicity of equal doses of VOCs given orally and by inhalation. Male S-D rats (300-375 g) were administered 179 mg CCl_4/kg bw as an aqueous Emulphor® emulsion, either as a single oral bolus by gavage or over 2 hours by constant gastric infusion via an indwelling gastric cannula. Groups of rats were sacrificed by etherization at time-intervals during and after administration of CCl_4. Blood was collected by cardiac puncture and selected tissues (i.e., liver, kidney, skeletal muscle, fat, lung, heart and brain) excised. The blood and tissues were processed for determination of CCl_4 content by GC headspace analysis. SDH and GPT activities were measured in the serum as indicators of hepatotoxicity (Sanzgiri *et al.*, 1992b).

Our results indicate that the PATTERN of ingestion (i.e., oral dosage regimen) substantially influences the uptake and tissue distribution of CCl_4, especially in the liver. The highest CCl_4 concentration was seen in the liver at the earliest sampling time (i.e., 1 minute) after oral dosing. The liver exhibited the highest concentrations of CCl_4 of any tissue during the first 15 minutes after bolus dosing. In contrast, liver levels of CCl_4 were the lowest of any tissue at each time-point in the gastric infusion group. This likely reflects the high capacity of the liver to metabolize CCl_4 and many other chemicals. When given as a single oral bolus, the relatively large amounts of CCl_4 entering the liver appeared to exceeded its metabolic capacity. Despite the high hepatic concentrations of CCl_4 during the first 15 minutes after bolus gavage, the liver consistently exhibited the lowest levels of CCl_4 of any tissue from 1 to 24 hours post dosing. Given time, the liver was apparently able to metabolize and eliminate much of its burden of the chemical. Although the fat

was the slowest to take up CCl_4, it accumulated very high concentrations of CCl_4 and showed the slowest clearance of the chemical.

CCl_4 was more hepatotoxic when given as a single oral bolus than when given by constant gastric infusion (Sanzgiri *et al.*, 1992b). Serum enzyme activities in both groups were not significantly elevated over controls until 8 hours post dosing. Thereafter, enzyme activities continued to increase for up to 24 hours. Thus, there was a substantial lag time between the uptake of CCl_4 by the liver and other organs and increases in serum SDH and GPT activities. These two enzymes are known to be released into the bloodstream from hepatocytes with cell membranes which are no longer intact. Hence, serum enzymes are of limited utility for correlation with chemical uptake and activation to cytotoxic metabolites. There have been reports of changes in other biochemical parameters in the liver within minutes of CCl_4 exposure. Thus, other more sensitive indices of hepatic cytotoxicity must be used to correlate with the time-courses of tissue deposition and metabolic activation of CCl_4.

As it was necessary to use relatively high (i.e., 1-5%) concentrations of Emulphor® to produce stable aqueous emulsions of CCl_4 in the foregoing studies, we conducted an investigation of potential effects of the emulsifier on CCl_4 pharmacokinetics and hepatotoxicity. Doses of 10 and 180 mg CCl_4/kg bw were given by gavage to fasted male S-D rats as an aqueous emulsion, using 1, 2.5, 5 and 10% Emulphor®. Serial blood samples were taken at intervals of 2 to 60 minutes for up to 12 hours, in order to obtain blood CCl_4 concentration versus time profiles. The animals were sacrificed 24 hours after dosing and blood taken for assay of serum enzymes as indices of hepatotoxicity. There were no significant differences in any pharmacokinetic parameter as a function of Emulphor® concentration. Similarly, the hepatotoxic potency of CCl_4 was not dependent upon the concentration of the emulsifying agent. It can be concluded from these results that Emulphor®, in the concentration range of 1 to 10% in oral dosing solutions, does not significantly alter the absorption, disposition or hepatotoxicity of CCl_4. These findings were presented to the Federation of American Societies for Experimental Biology (Sanzgiri *et al.*, 1991b) and are included in a manuscript which is now being written.

ADDITIONAL studies are needed to clarify the question of whether target organ toxicity is independent of ROUTE of exposure. Our results to date clearly demonstrate that route of exposure has a pronounced effect on the pharmacokinetics of VOCs such as CCl_4. The marked differences in blood levels should result in marked differences in the quantity of CCl_4 deposited in tissues, and in turn the extent of toxic injury of the tissue. Unfortunately, there are few data on tissue uptake of inhaled or ingested VOCs. Our only toxicity data point was 24 hours post exposure. CCl_4 and other VOCs are known to be very rapidly absorbed and available for distribution to tissues. Furthermore, it is known that CCl_4 can exert cytotoxic effects within minutes after dosing. Thus, "minute-by-minute" data are needed on the relative tissue uptake, metabolic activation (to toxic metabolites), and toxicity of INHALED versus INGESTED VOCs. This information should provide answers to the previously posed questions/hypotheses that: (a) the liver is at greater risk (of toxicity and cancer) from ingestion of VOCs; and (b) extrahepatic organs are at significantly greater risk from inhalation of VOCs.

B. Characterization of Presystemic Elimination of Ingested VOCs

One of the major objectives of the project was to determine the relative significance of FIRST PASS elimination of ingested halocarbons by the liver and lungs. Although there was very limited information about the role of "first pass" uptake, it seemed likely that substantial proportions of some VOCs absorbed from the GI tract will be metabolically degraded and/or exhaled before reaching the systemic circulation and extrahepatic target organs. Relatively low doses, as are encountered ENVIRONMENTALLY, should be most significantly affected.

In light of the foregoing, experiments were undertaken to elucidate the influence of oral dosage regimen on the systemic uptake, disposition and elimination of a series of halocarbons. Although all of the halocarbons selected for study were volatile, two were extensively metabolized (i.e., 1,1,2-trichloroethylene (TCE) and 1,1-dichloroethylene (1,1-DCE)), and two were poorly metabolized (1,1,1-trichloroethane (TRI) and perchloroethylene (PER)). Although patterns of water consumption vary in human populations, constant gastric infusion was selected to approximate frequent, repetitive ingestion of water. Equivalent doses of each halocarbon were given to unanesthetized, male S-D rats as an aqueous Emulphor® emulsion, either orally as a single bolus or infused through a surgically-implanted gastric cannula for 2 hours. The animals were surgically prepared the day before by implantation of a cannula into their right carotid artery, so that serial micro-blood samples could subsequently be taken from unanesthetized, freely-moving animals. Periodic blood samples of 25-50 μl were taken via the carotid cannula and analyzed for halocarbon content by a headspace technique in a gas chromatograph equipped with an electron capture detector. Blood concentration versus time profiles were thereby obtained and a variety of pharmacokinetic parameters determined.

Data analysis revealed marked pharmacokinetic differences between the oral bolus and intragastric (ig) infusion groups. Significant reductions in area under the blood concentration versus time curve (AUC) and peak blood concentration (C_{max}), and increased (blood) elimination half-life ($t_{½}$) values were observed for each halocarbon, when it was given by ig infusion (versus bolus dosing). Blood concentrations of the two well metabolized halocarbons studied, TCE and 1,1-DCE, were so low that they were hardly detectable at low dosage levels in the ig animals. These findings may be very important implications in toxicity and cancer risk assessment, in that the liver and lungs may be able to remove virtually ALL of the trace amounts of VOCs that are normally found in drinking water and foods. Thus, trace levels of VOCs should NOT pose a significant CANCER risk, even under the "worst case" assumptions used by the EPA in its cancer risk calculations.

The aforementioned findings were presented at the AFOSR symposium at the 1990 meeting of the Society of Environmental Toxicology and Chemistry (SETAC) meeting (Bruckner *et al.*, 1990b). Two publications are in draft form and will be submitted for publication this year.

In a recently completed investigation (Lee *et al.*, 1991), a comprehensive evaluation of the (maximum) capacity and dose-dependency of presystemic elimination of TCE was performed. Direct measurement studies, in groups of animals given the VOC at different intravascular sites, appeared to be an

expeditious way to accurately assess the relative contributions of the liver and lungs to presystemic elimination. A series of equivalent doses of TCE were administered via the portal vein (through a surgically implanted cannula), intravenously (iv), intraarterially (ia) and orally (po). Determination of the area under the blood concentration time curves (AUCs) for each administration site allowed the fractional contribution of each organ to first pass effects to be determined.

The following intravascular administration and blood sampling sites were utilized:

Exposure route	Administration site	Sampling site
iv	jugular vein	carotid artery
po	stomach via gastric tube	carotid artery
portal vein	portal vein	carotid artery
ia	carotid artery	femoral artery

Bioavailability (F) represents the fraction of the dose that reaches the systemic circulation. It can be expressed as: $F = f_g f_h f_l$ where f_g = the fraction of dose escaping GI metabolism or elimination, f_h = the fraction of dose escaping hepatic metabolism or elimination, and f_l = the fraction of dose escaping lung metabolism or elimination. These fractional components of F can be assessed as follows: $f_g = (AUC)_{po}/(AUC)_{pv}$, $f_h = (AUC)_{pv}/(AUC)_{iv}$, and $f_l = (AUC)_{iv}/(AUC)_{ia}$ where $(AUC)_{po}$ = the area under the blood VOC concentration-time curve from time zero to infinity following oral administration, $(AUC)_{pv}$ = the area under the blood VOC concentration-time curve from time zero to infinity following portal vein injection, $(AUC)_{iv}$ = the area under the blood VOC concentration-time curve from time zero to infinity following jugular vein injection, and $(AUC)_{ia}$ = the area under the blood VOC concentration-time curve from time zero to infinity following carotid artery injection. The product of F_g, f_h, and f_l is equal to $(AUC)_{po}/(AUC)_{ia}$ or F. By evaluating F_g, f_h and f_l therefore F as a function of dose, the extent and source of saturable presystemic elimination of VOCs can be ascertained.

Using the above approach, cannulas are surgically implanted into the hepatic portal vein (PV), jugular vein and/or carotid artery of male S-D rats of 330-380 g. After the animals recover for 48 hours, one of a series of doses of TCE is injected over 30 seconds intravascularly and serial micro-blood samples collected for TCE analysis. The injection and sampling sites determine whether first-pass hepatic or pulmonary elimination is measured, as described above. Non-linearity of TCE elimination of PV doses higher than 7 mg/kg was manifest by diminished clearance values. The higher doses of TCE exceeded the metabolic capacity of the animals. Total presystemic elimination was inversely related to dose, ranging from >70% at 0.65 mg/kg to <10% at 13.6 mg/kg and higher. The fraction of the administered dose eliminated by the lungs remained fairly consistent (i.e., 10-16% over the range of doses administered. In contrast, the fraction eliminated by the liver increased as the dose diminished. First-pass hepatic elimination accounted for 51% of the lowest dose (0.65 mg/kg) (Lee *et*

al., 1991). Administration of even lower doses over a longer time-frame (as would occur under actual environmental exposure conditions) should result in even greater presystemic elimination.

We have recently conducted experiments to assess the influence of dosage RATE on the efficiency of presystemic elimination of low doses of TCE. Total presystemic elimination of a 0.3 mg/kg dose, injected intravascularly over 30 seconds, was quite similar to that of the 0.65 mg/kg dose (i.e., ~70%). It was not possible to give lower doses, as our analytical method will not accurately quantify TCE in blood during the latter part of the terminal elimination phase (when TCE concentrations drop below 1 ng). Thus, based upon our results to date, it appears that a constant percentage (i.e., ~70%) of low, bolus (in this case, administered over 30 seconds) doses are eliminated by the liver and lungs. If the chemical were consumed more gradually (i.e., over a more prolonged period), it would appear that first pass uptake would remove a higher percentage. Indeed, when the 0.65 mg/kg dose of TCE is infused over 30 minutes to 1 hour, the VOC is not detectable in the arterial blood. Thus, trace amounts of VOCs consumed in drinking water over the course of the day may be COMPLETELY removed, and NOT pose a health risk to extrahepatic organs.

ADDITIONAL RESEARCH is needed to provide a more complete data base to test this important hypothesis above. More sensitive analytical techniques should be developed to accurately quantify < ng concentrations of VOCs in biological samples. Additional VOCs with different physical/chemical characteristics should be studied, using the experimental approach described above. VOC mixtures should also be studied, since multiple VOCs are commonly found in contaminated drinking water. Finally, it would be worthwhile to investigate VOCs which destroy drug metabolizing enzymes and/or kill liver cells. Such chemicals, in sufficient doses, should inhibit their own metabolism and presystemic elimination.

C. Investigation of the Role of the Lymphatics in GI Absorption of VOCs and the Influence of Corn Oil on GI Absorption Pathways

Although the LYMPHATICS represent a major pathway for absorption of long-chain dietary fatty acids, there is very little information about the role of the lymphatics in GI absorption of VOCs and other hydrocarbons. It is possible that ingestion of VOCs in a digestible oil may result in their incorporation into chylomicrons and entry into the lacteals rather than the blood. If VOCs do enter the lymphatic vessels rather than the portal blood, the chemicals will not be subject to first pass metabolism and elimination. Also, certain chemicals may exert immunotoxic effects within the lymphatic system.

An investigation was conducted to: (a) determine whether an orally administered halocarbon (i.e., CCl_4) was absorbed via the lymphatics; (b) evaluate the relative importance of the lymphatics versus the portal (blood) vasculature in GI absorption; (c) examine the influence of oil dosing vehicles on the absorption pathways. Male S-D rats were surgically prepared with indwelling thoracic duct and jugular vein cannulas. The thoracic duct is the major lymphatic vessel in the body. The distal ends of the two cannulas were exteriorized through the same incision and connected, in order to reestablish the return of the lymph to the venous circulation during the animal's 24-hour recovery period. Each cannulated rat was administered CCl_4 of 99+% purity as a

single oral bolus of 25 mg/kg: (a) in corn oil; (b) as an 0.5% aqueous Emulphor® emulsion; and (c) in water. Lymph was collected under toluene continuously for up to 10 hours after dosing. Serial venous blood samples were taken during this time period from another group of animals. The CCl_4 content of the blood and lymph samples was measured by gas chromatography headspace analysis.

Data from the foregoing absorption pathways study revealed that orally administered CCl_4 was absorbed concurrently into the lymphatic and the venous circulatory systems. The proportion of chemical absorbed via the lymphatics was quite small, relative to that taken up into the portal venous blood. The dosage vehicle/diluent, however, had a pronounced effect on the absorption pathways. Approximately 0.75% of the 25 mg/kg dose was estimated to be absorbed via the lymphatics, when it was given in corn oil, versus only 0.043% and 0.058% for the emulsion and water groups, respectively. The CCl_4 concentration was 10- to 30-fold higher in the lymph of the corn oil animals than in that of the emulsion and water animals. Moreover, the CCl_4 concentration in the corn oil group was 10- to 100-fold higher in lymph than in the blood during the 10-hour monitoring period. This phenomenon may have important implications in the interpretation of immunology and cancer bioassays, in which VOCs and other organic chemicals have been routinely administered to rodents in oil vehicles. The relatively high chemical concentrations in the lymph may suppress immune function, possibly resulting in increased expression of chemically-induced cell mutations and cancers.

The lymph and blood CCl_4 concentration versus time profiles of rats given the chemical in corn oil were quite different, qualitatively as well as quantitatively, from profiles in the aqueous vehicle groups. CCl_4 levels increased very quickly following dosing in both venous blood and lymph in the water and emulsion groups, indicating rapid absorption of the halocarbon from the GI tract. Concentrations of CCl_4 increased and decreased more slowly in the lymph than in the blood during the 10-hour post-exposure period in both aqueous vehicle groups. CCl_4 levels in lymph and blood exhibited prolonged elevation, with multiple secondary peaks. This "plateau phenomenon" can be ascribed to the marked delay and prolongation of CCl_4 absorption from corn oil seen in a previous study (Kim *et al.*, 1990b). Despite the prolonged, relatively high concentrations of CCl_4 in lymph, the portal (venous) blood accounted for the majority of CCl_4 absorption. This can be attributed to the markedly greater flow rate in the portal circulation. Results of this investigation were published in abstract form (Kim *et al.*, 1990d), and are being incorporated into a paper to be submitted for publication this fall.

ADDITIONAL RESEARCH in the future should be conducted to elucidate the relative significance of LYMPHATIC versus portal VENOUS absorption of ingested VOCs. As described above, a fraction of the orally administered dose of CCl_4 is absorbed into the lymphatics of rats. Use of a corn oil vehicle substantially increases the proportions of the dose entering the lymphatic system. It would be anticipated that the LIPID SOLUBILITY of a chemical will determine its absorption pathway(s). Chemicals with high lipid solubility (e.g., PCBs, dioxin, DDT) may be absorbed predominantly via the lymphatics. As alluded to above, substantial concentrations of certain chemicals in the lymph may bypass presystemic elimination and suppress immune function, which may in turn result in increased expression of chemically-induced mutations and cancers. It can also

be hypothesized that organic chemicals will be absorbed from the gut via alternate pathways, depending upon the vehicle/diluent in which they are ingested. Therefore, it would be worthwhile to investigate the GI absorption pathways and immunosuppressive potency of a series of chemicals of varying lipophilicity given in aqueous and oil vehicles.

D. Characterization of the Influence of Oral Dosage Vehicles on the Absorption, Pharmacokinetics and Toxicity of VOCs

A large number of VOCs are of major health concern as drinking water contaminants. It has been necessary in most oral toxicity studies to give the VOCs in OIL dosage vehicles, due to their limited water solubility. This routine use of oil-based vehicles may introduce confounding factors, which could substantially affect the relevancy of study results to risk assessment of VOCs in drinking water.

The objective of the first series of studies we undertook was to assess the influence of dosing vehicles on the pharmacokinetics and the acute hepatotoxicity of CCl_4. In one experiment, fasted male S-D rats were given a series of doses of CCl_4 by gavage: in corn oil; as an aqueous Emulphor® emulsion; as the undiluted chemical; and in water. Blood and liver samples were taken 24 hours post dosing for measurement of serum and hepatic microsomal enzymes and for histopathological examination. Acute hepatotoxicity, as reflected by these parameters, was less pronounced at each dosage level in rats given CCl_4 in corn oil than in the other vehicles. In contrast, the aqueous emulsion did not substantially alter the toxicity of CCl_4 from that of undiluted CCl_4 or CCl_4 ingested in water.

In the companion experiment, rats received a single dose (25 mg/kg) of CCl_4 by gavage: in corn oil; as an aqueous Emulphor® emulsion; as the undiluted chemical; and in water. The 25 mg/kg dose was given to a second group of rats intravenously (iv) through an indwelling jugular cannula. Serial blood samples were taken from both groups via a surgically-implanted carotid artery cannula and analyzed for CCl_4 content by headspace gas chromatography, in order to obtain blood concentration versus time profiles. CCl_4 was absorbed very rapidly from the GI tract, as peak blood concentrations were reached within 3 to 6 minutes after dosing in the aqueous emulsion and water groups. These peak levels were much higher than in the corn oil group. Corn oil markedly delayed the absorption of CCl_4 from the GI tract and produced secondary peaks in the blood profiles. Elimination from the bloodstream of the iv group followed a triexponential pattern. There was a high degree of correlation of both C_{max} and AUC^{120} with the magnitude of hepatotoxicity. CCl_4 was less toxic in corn oil due to delay and prolongation of CCl_4 absorption, resulting in a marked decrease in CCl_4 concentration in the arterial blood and likely in the liver. These findings demonstrate that corn oil has sufficient effect on the pharmacokinetics and acute hepatotoxicity of CCl_4 to warrant REAPPRAISAL of the use of oil dosage vehicles in toxicity studies of VOCs. The use of aqueous Emulphor® emulsions, however, appears appropriate in studies of VOC contaminants of drinking water, in that the emulsion did not substantially alter the PK or toxicity from that of CCl_4 ingested in water. These findings were published in two papers (Kim *et al.*, 1990a & b).

Effort was also devoted to pharmacokinetic analysis and computer modeling of the GI absorption of VOCs from oil diluents. Ramsey and Andersen (TAP 73:159-175, 1974), in a "benchmark" paper in physiologically-based pharmacokinetic (PBPK) modeling of VOCs, could not model the GI uptake and disposition for styrene from vegetable oil. They concluded that more complex modeling efforts would be required to simulate and predict the effect of oils on the systemic absorption of VOCs. A system analysis approach was thus used to analyze the data from the CCl_4 pharmacokinetic studies described in the preceding paragraph. System analysis makes use of the convolution-deconvolution relationship to describe the output and input into a system. Unlike classical compartmental modeling approaches that typically assume first- or zero-order absorption processes, the emphasis in system analysis is on obtaining a function that characterizes the system outputs (i.e., blood concentration versus time data). Estimation of the cumulative percent of CCl_4 absorbed over time by deconvolution indicated that corn oil would result in prolonged, erratic absorption of CCl_4. This was indeed the case, as evidenced in the blood profiles of animals dosed with CCl_4 in corn oil by a long T_{max} (time of maximum blood level) and pronounced intersubject variability. The system analysis modeling work was presented at an EPA- and ILSI-sponsored risk assessment conference in 1990, and published the same year (Gillespie *et al.*, 1990). Subsequent work was done to evaluate the usefulness of linear system analysis as an adjunct to PBPK modeling. A PBPK model, employing an absorption input rate function provided by system analysis, accurately predicted blood CCl_4 concentration-time date for both aqueous and oil oral dosage vehicles. This is the first time, to our knowledge, that the uptake and disposition of a VOC from an oil vehicle has been successfully modeled. These findings were incorporated into a manuscript which recently accepted for publication (Gallo *et al.*, 1992).

Studies by several groups of investigators have shown that the oral subchronic toxicity and carcinogenicity of VOCs can be significantly altered by the gavage vehicle. Chloroform ($CHCl_3$) and a number of other halocarbons have been found to produce a very high incidence of hepatocellular carcinoma, when given to B6C3F1 mice in corn oil by gavage. Jorgenson *et al.* (*FAAT 5:*760-769, 1985), however, saw no evidence of tumorigenesis when these mice were given the same doses of $CHCl_3$ in drinking water. Similarly, Klaunig *et al.* (*Envir. Health Perspec. 69:*89-95, 1986) found that $CHCl_3$, 1,1-DCE and 1,2-dichloroethane were not carcinogenic when given to mice in their drinking water, although each VOC was reported to be a hepatocarcinogen when administered by gavage in corn oil. CCl_4 and $CHCl_3$ have been reported by other researchers to be more hepatotoxic when given to mice for 90 days in corn oil, than when given in an aqueous suspension. We conducted two subchronic studies of the hepatotoxicity of CCl_4 given orally to rats in corn oil versus aqueous vehicles. In the first, a battery of indices consistently indicated CCl_4 to be somewhat more hepatotoxic after 2 and 4 weeks of dosing when given in corn oil. In the second study, CCl_4 given in corn oil was slightly more hepatotoxic at 4 and 8 weeks, but liver injury was of similar magnitude after 13 weeks in the corn oil and aqueous vehicle groups. It would appear that the aforementioned dissimilarities in hepatotoxic and carcinogenic potency of halocarbons in different studies may be attributable to differences in both dosage vehicle and dosage regimen (i.e., bolus versus divided doses). The results of one of these studies were presented to the Society of Toxicology (Koporec *et al.*, 1990). The findings of this study

were included in a manuscript just submitted for publication (Koporec *et al.*, 1992). A manuscript based on the second study is presently being drafted.

E. Investigation of Potential Mechanisms by Which Corn Oil Alters the Pharmacokinetics and Toxicity of Orally Administered VOCs

The final objective of this project was to examine possible MECHANISMS by which CORN OIL affects the pharmacokinetics and toxicity of ingested VOCs. Studies have already been discussed which clarify some of the mechanisms of alteration of the pharmacokinetics and toxicity of a representative halocarbon, CCl_4. Corn oil delayed and prolonged the GI absorption of CCl_4, resulting in a significant decrease in its acute hepatotoxicity, but some increase in toxicity during the initial weeks of subchronic CCl_4 exposure. Elevated lipid intake from corn oil caused increased deposition of triglycerides in the liver, which in turn resulted in increased deposition of CCl_4. Although it has been suggested that high dietary corn oil may enhance lipoperoxidation in liver microsomes and mitochondria, as well as increase the superoxide and peroxide content of the liver, our first subchronic study showed that corn oil did not cause an increase in hepatic microsomal lipid peroxidation in CCl_4-treated or vehicle control animals.

An investigation in our laboratory revealed that dietary polyunsaturated fatty acids are needed for optimal activity and induction of hepatic microsomal cytochrome P-450 in rats. Male S-D rats were starved for 36 hours, and then refed a fat-free (FF) diet or a diet containing 20% corn oil (COD) for 4 days. Some rats were dosed with phenobarbital (PB) daily for 3 days during this period. PB-treated FF animals had only 21% more P-450 than FF controls, whereas rats fed the 20% COD had 59% more P-450, and the PB-treated COD rats had 181% more P-450 than the FF controls. Five P-450 isozymes separated by SDS-PAGE were quantified using a gel scanner. Analysis of the gels showed 32, 59, and 124% more P-450 (total isozymes) in FF PB, COD, and COD PB groups, respectively, than in the FF groups. These findings suggest that dietary fat deprivation reduces the total amount of cytochrome P-450 hemoprotein and its inductibility by PB through decreased P-450 hemoprotein synthesis. The limiting factor(s) restricting synthesis of new cytochrome P-450 hemoprotein in rats devoid of fat may be an inability to respond to the inducer (PB), or the deficit of fatty acids needed for synthesis of the phospholipid matrix of the microsomal membrane necessary for support and proper juxtapositioning of these enzymes. These findings were incorporated into a manuscript which was published (Kim *et al.*, 1990c).

A study was initiated to evaluate the effect of dietary fat deprivation on the acute toxicity of CCl_4. Male S-D rats were starved for 36 hours and then refed a FF diet or a 20% corn oil diet (COD) for 4 days. Some animals received phenobarbital (PB) sodium (80 mg/kg, ip daily) for 3 days. One half of the PB-treated and control rats were given 250 mg CCl_4/kg bw. Blood and liver samples were taken 24 hours after dosing for measurement of serum and microsomal enzymes and histopathological examination. Hepatic microsomal proteins were separated by SDS-PAGE and P-450 isozymes quantitated with a gel scanner. Significantly greater damage to hepatocytes was evident in rats fed the COD by elevated serum enzyme levels and histologic changes. Whereas CCl_4 produced no destruction of P-450 in FF rats, it decreased P-450 levels in COD rats by nearly 50%. CCl_4 reduced P-450 160% in COD-PB rats. CCl_4 significantly reduced P-450 isozymes in

COD rats, while having no effect on isozyme levels of FF rats. It was possible to gain insight into the role of different P-450 isozymes in the bioactivation of CCl_4 (to cytotoxic metabolites), by correlation of the differential effects of the FF diet, COD and PB on individual P-450 isozymes and the extent of CCl_4-induced liver injury. Two isozymes (i.e., 51.2 and 53 kd components), in particular, were induced by PB and appeared to be active in potentiation of CCl_4 hepatotoxicity by PB. These two constitutive forms of P-450, known collectively as the PB-B form of the enzyme, seemed to be primarily responsible for metabolic activation of CCl_4. These findings were published in abstract form (Choi *et al.*, 1990) and have been included in a manuscript to be submitted for publication this year.

One of the stated aims of the project is to test the hypothesis that corn oil enhances the elimination (and toxicity) of VOCs by induction of liver mixed-function oxidase (MFO) activity. Although we have demonstrated that dietary lipids are required for synthesis of P-450 hemoproteins and metabolism of xenobiotics, it was not clear whether a substantial increase in lipid intake would produce an increase in P-450 levels and metabolic capacity over what is usually present in animals consuming a normal diet. This question has important implications. VOCs are typically given to animals by corn oil gavage in toxicology and cancer studies. If the high doses of corn oil induce P-450 and MFO activities, the metabolism and toxicity or carcinogenicity of the test chemical may be significantly altered.

We undertook a study of the influence of corn oil (CO) ingestion on hepatic microsomal cytochrome P-450 and its isozymes. The objectives of the study were to determine whether short- and long-term, high-level corn oil ingestion caused: (a) a change in total P-450 levels; (b) an alteration in the profile of major P-450 isozymes; and (c) a difference in the metabolism of selected chemicals. Male S-D rats were given 5 ml CO/kg bw daily by gavage for up to 13 weeks. Groups of 5 rats were sacrificed at 1 day and at 1, 2, 4, 8, and 13 weeks. The liver was removed and total microsomal cytochrome P-450 measured spectrophotometrically. Hepatic microsomal proteins were separated by SDS-PAGE and P-450 isozymes quantitated with a gel scanner. Benzo(a)pyrene hydroxylase, ethylmorphine demethylase (EMD) and N-nitrosodimethylamine demethylase (NDMD) activities were measured by standard techniques. There were no significant differences between CO and control groups in total P-450 or in constitutive P-450 isozymes during the 13-weeks. MFO activities occasionally appeared somewhat lower in the CO animals during the period, but only at 13 weeks were the decreases from controls in EMD and NDMD activities statistically significant. Thus, excessive CO intake does not appear to induce, nor to substantially alter hepatic P-450s or mixed function oxidase activity. These findings were presented to the Society for Pharmacology and Experimental Therapeutics (Kim and Bruckner, 1990), and will be submitted for publication this summer.

IV. ABSTRACTS AND JOURNAL ARTICLES

Bruckner, J.V., Kim, H.J., Muralidhara, S., and Gallo, J.M. Influence of route and pattern of exposure on the pharmacokinetics and hepatotoxicity of carbon tetrachloride. In: Principles of Route-to-Route Extrapolation for Risk Assessment, Gerrity TR and Henry CJ, eds., pp. 271-284, Elsevier, New York (1990a).

Bruckner, J.V., Dallas, C.E., Muralidhara, S., Srivatsan, V., Manning, R.O., and Gallo, J.M. Presystemic elimination of volatile organic compounds (VOCs) ingested in water. Society of Environmental Toxicology and Chemistry 11:240 (1990b).

Choi, C.W., Kim, H.J., Jun, H.W., Voss, K.A., Bruckner, J.V., and Wade, A.E.: Alteration of carbon tetrachloride (CCl_4) hepatotoxicity by dietary fat. Toxicologist 10:198 (1990).

Gallo, J.M., Cheung, L.L., Kim, H.J., Bruckner, J.V., and Gillespie, W.R. Physiological pharmacokinetic model for carbon tetrachloride: Use of oral absorption input functions obtained by system analysis. Accepted for publication in Toxicol. Appl. Pharmacol. (1992).

Gillespie, W.R., Cheung, L.L., Kim, H.J., Bruckner, J.V., and Gallo, J.M.: Application of system analysis to toxicology: Characterization of carbon tetrachloride oral absorption kinetics. In: Principles of Route-to-Route Extrapolation for Risk Assessment, Gerrity, T.R. and Henry, C.J., eds., pp. 285-295, Elsevier, New York (1990).

Kim, H.J., Odend'hal, S., and Bruckner, J.V.: Effect of oral dosing vehicles on acute hepatotoxicity of carbon tetrachloride in rats. Toxicol. Appl. Pharmacol. 102:34-49 (1990a).

Kim, H.J., Gallo, J.M., Dallas, C.E., and Bruckner, J.V.: Effect of oral dosing vehicles on the pharmacokinetics of orally administered carbon tetrachloride in rats. Toxicol. Appl. Pharmacol. 102:50-60 (1990b).

Kim, H.J., Choi, E.S., and Wade, A.E. Effect of dietary fat on the induction of hepatic microsomal P-450 isozymes by phenobarbital. Biochem. Pharmacol. 39:1423-1430 (1990c).

Kim, H.J., Muralidhara, S., Choi, C.W., and Bruckner, J.V. Role of the lymphatics in absorption of carbon tetrachloride (CCl_4) given orally in different dosing vehicles. Toxicologist 10:52 (1990d).

Kim, C. and Bruckner, J.V. Effect of increased corn oil ingestion on hepatic microsomal cytotochrome P-450 and mixed-function oxidase (MFO) activity. Pharmacologist 32:174 (1990).

Koporec, K.P., Kim, H.J., MacKenzie, W.F., and Bruckner, J.V. Evaluation of oral dosing vehicle effects on the subchronic hepatotoxicity of carbon tetrachloride (CCl_4) in the rat. Toxicologist 10:52 (1990).

Koporec, K.P., Kim, H.J., MacKenzie, W.F., and Bruckner, J.V. Effect of oral dosing vehicle on the subchronic hepatotoxicity of carbon tetrachloride in the rat. Submitted for publication (1992).

Lee, K., Muralidhara, S., and Bruckner, J.V. Presystemic elimination of trichloroethylene (TCE); Direct assessment using an unanesthetized rat model. Pharmacologist 33:178 (1991).

Manning, R.O., Brown, K.H., Srivatsan, V., Gallo, J.M., and Bruckner, J.V. Pharmacokinetics of trans-1,2-dichloroethylene (DCE) and 1,1-dichloroethane (DCA) in rats. Toxicologist 10:235 (1990).

Sanzgiri, U.Y., Muralidhara, S., Dallas, C.E., and Bruckner, J.V. Effect of route of administration on the pharmacokinetics and acute hepatotoxicity of carbon tetrachloride (CCl_4). Toxicologist 11:173 (1991a).

Sanzgiri, U.Y., Muralidhara, S., Dallas, C.E., and Bruckner, J.V. Effet of route of administration on the pharmacokinetics and acute hepatotoxicity of carbon tetrachloride (CCl_4). FASEB Journal 6:A1570 (1991b).

Sanzgiri, U.Y., Kim, H.J., Dallas, C.E., Muralidhara, S., and Bruckner, J.V. Influence of route and pattern of exposure on target organ toxicity and pharmacokinetics of carbon tetrachloride in rats. Submitted for publication (1992a).

Sanzgiri, U.Y., Muralidhara, S., and Bruckner, J.V. Correlation of tissue distribution and hepatotoxicity of carbon tetrachloride (CCl_4) following ingestion. Toxicologist 12:423 (1992b).

V. PROFESSIONAL ACTIVITIES RELATED TO PROJECT TOPICS

Drs. Bruckner and Gallo presented a joint seminar on the pharmacokinetics (PK) and physiologically-based PK modeling of volatile organic compounds to the U.S. EPA Health Effects Research Laboratory in Research Triangle Park, NC, February, 1989.

Dr. Bruckner served as chairman of a scientific session on halogenated hydroarbons at the national Society of Toxicology Meeting in Atlanta, GA, March, 1989.

Drs. Bruckner, Dallas, Gallo and Kim and Mr. Muralidhara attended and presented research papers at the Society of Toxicology Meeting in Atlanta, GA, March, 1989.

Dr. Bruckner presented a seminar on factors which influence halogenated hydrocarbon toxicity at the U.S. EPA Health Effects Research Laboratory in Research Triangle Park, NC, February, 1990.

Drs. Bruckner, Dallas, Hyo, and Manning and Mr. Muralidhara attended and presented research papers at the annual meeting of the Society of Toxicology in Miami, FL, February, 1990.

Drs. Bruckner and Gallo presented invited papers at the Principles of Route-to-Route Extrapolation for Risk Assessment Workshop, sponsored by the U.S. EPA and ILSI, Hilton Head, SC, March, 1990.

Dr. Gallo attended and made a presentation on physiologically-based pharmacokinetic modeling at the 23rd annual Higuchi Research Conference in Ozark, MO, March, 1990.

Drs. Bruckner and Dallas each gave invited research presentations at a symposium on AFOSR-sponsored research at the 11th annual meeting of the Society of Environmental Toxicology and Chemistry in Arlington, VA, November, 1990.

Drs. Bruckner, Dallas, Manning, and Srivatsan and Mr. Muralidhara attended and presented research papers at the Society of Toxicology Meeting in Dallas, TX, February, 1991.

Dr. Bruckner served as chairman of a scientific session on halogenated hydrocarbons at the annual Society of Toxicology Meeting in Dallas, TX, March, 1991.

Drs. Bruckner and Gallo gave a joint research presentation entitled "Characterization and Physiological Modeling of the Pharmacokinetics of Volatile Organic Chemicals" to the Environmental Toxicology Division of the U.S. EPA Health Effects Research Laboratory, Research Triangle Park, NC, July, 1991.

Dr. Bruckner served as a reviewer for Toxicological Profiles a number of volatile organic chemicals, including 1,2-dibromo-3-chloropropane, trichloroethylene, carbon tetrachloride, 1,1-dichloroethane and 1,1,2-trichloroethane for the U.S. Agency for Toxic Substances and Disease Registry and the U.S. EPA.

Dr. Bruckner served on an expert panel on pharmacokinetics of chemical mixtures for the Exposure Assessment Group, Office of Health and Environmental Assessment, U.S. EPA.

Dr. Bruckner served on a working group on chemical contaminants in reclaimed water for the space station, National Academy of Sciences and the National Aeornautics and Space Administration.

Dr. Bruckner served as a consultant to Shell, Occidental, Exxon and other companies having problems with groundwater contamination by volatile organic chemicals.

Dr. Bruckner is a member of the Committee on Pesticides in the Diets of Infants and children, for the Board on Agriculture and the Board on Environmental Studies and Toxicology, National Acedemy of Sciences.

Drs. Bruckner, Dallas, Gallo, and Manning serve as editorial board members and reviewers for scientific journals, in processing research manuscripts pertaining to the toxicity and pharmacokinetics of volatile organic chemicals and other compounds.

VI. ADVANCED DEGREES AWARDED

Hyo J. Kim, Ph.D., June, 1989. Effects of Oral Dosing Vehicles, Routes and Patterns of Exposure on Target Organ Toxicity and Pharmacokinetics of Carbon Tetrachloride in Rats.

Kevin P. Koporec, M.S., June, 1990. Effect of Oral Dosing Vehicles on the Subchornic Hepatotoxicity of Carbon Tetrachloride (CCl_4) in the Rat.

Uma Y. Sanzgiri, Ph.D., anticipated December, 1992. Influence of Route and Pattern of Exposure on the Toxicokinetics of Carbon Tetrachloride.

Monica K. Lee, Ph.D., anticipated August, 1993. Presystemic Elimination of Trichloroethylene and Other Volatile Organic Chemicals.